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APPLICATION NUMBER: ***60/579,533***
FILING DATE: ***June 14, 2004***
RELATED PCT APPLICATION NUMBER: ***PCT/US05/02556***

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV362100470US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Justin	Hanes	Baltimore, MD

Additional inventors are being named on the one *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Surface Modification of Particles

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☐ Customer Number:

OR

☑ Firm or Individual Name	Johns Hopkins University				
Address	100 N. Charles Street				
Address	5th Floor				
City	Baltimore	State	MD	Zip	21201
Country	USA	Telephone	410-516-8300	Fax	410-516-5113

ENCLOSED APPLICATION PARTS ***(check all that apply)***

☑ Specification *Number of Pages* 18

☐ Drawing(s) *Number of Sheets* ________

☐ Application Data Sheet. See 37 CFR 1.76

☐ CD(s), Number ________

☐ Other (specify) ________

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[Page 1 of 2]

Respectfully submitted,

SIGNATURE [signature]

TYPED or PRINTED NAME Heather Bakalyar, Ph.D.

TELEPHONE 410-516-8300

Date 11-June-04

REGISTRATION NO. 45,282
(if appropriate)
Docket Number: 4485

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Michelle	Dawson	Perry Hall, MD
Denis	Wirtz	Washington, D.C.
Jie	Fu	Baltimore, MD

[Page 2 of 2]

Number 2 of 2

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INVENTION INFORMATION

Title of Invention: Surface Modification of Particles

Name of Lead Inventor:

Last: Hanes First: Justin Degree: Ph.D.

Lead Inventor Information: [The Lead Inventor is the primary contact person for LTD on all matters associated with this Report of Invention, including processing, patent prosecution and licensing. For reasons of administrative efficiency, it is the responsibility of the Lead Inventor to keep all other JHU inventors named on this Report of Invention informed of the status of such matters.]

Title or Position: Associate Professor E-mail: Hanes@jhu.edu

School: Johns Hopkins University Department: Chemical and Biomolecular Engineering

Business phone: (410) 516 -3484 Business fax: (410) 516 -5510

Business address: 3400 N. Charles St.
Baltimore, MD 21218

Interdepartmental address: MD 221

Home phone number: (Home fax number: ( ) -

Home address: 5416 Purlington Way, Baltimore, MD 21212

Citizenship: USA Social Security Number:

Are you a Howard Hughes Medical Institute employee or investigator? ☐ Yes ☒ No
Are you a Kennedy Krieger Institute employee or investigator? ☐ Yes ☒ No

Additional inventors: ☒ Yes ☐ No If yes, please complete Additional Inventors section for each inventor.

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ADDITIONAL INVENTOR(S)

Please copy this page for additional inventors as necessary

Name of Inventor:

Last Dawson First Michelle Degree B.S.

Title or Position: Ph.D. Candidate E-mail: Mdawson@jhu.edu

School: Johns Hopkins University Department: Chemical and Biomolecular Engineering

Business phone: (410) 516 - 5283 Business fax: (410) 516 - 5510

Business address: 3400 N. Charles St.
Baltimore, MD 21218

Interdepartmental address: Maryland Hall 221

Home phone number: (Home fax number: ( ) -

Home address: 1 Capland Ct.
Perry Hall, MD 21128

Citizenship: United States Social Security Number:

Are you a Howard Hughes Medical Institute employee or investigator? ☐ Yes ☒ No
Are you a Kennedy Krieger Institute employee or investigator? ☐ Yes ☒ No

Name of Inventor:

Last: Wirtz First : Denis Degree: Ph.D.

Title or Position: Professor E-mail: Wirtz@jhu.edu

School: Johns Hopkins University Department: Chemical and Biomolecular Engineering

Business phone: (410) 516 - 7006 Business fax: (410) 516 - 5510

Business address: 3400 N. Charles St.
Baltimore, MD 21218

Interdepartmental address: MD 221

Home phone number: (Home fax number: (

Home address: 3818 Gasrrison St. NW, Washington, DC 20016

Citizenship: Belgian Social Security Number:

Are you a Howard Hughes Medical Institute employee or investigator? ☐ Yes ☒ No
Are you a Kennedy Krieger Institute employee or investigator? ☐ Yes ☒ No

Name of Inventor: Last: Fu	First: Jie	Degree: Ph.D.
Title or Position: Research Scientist	E-mail: Jie@jhu.edu	
School: Johns Hopkins University	Department: Chemical and Biomolecular Engineering	
Business phone: (410) 516 - 3394	Business fax: (410) 516 - 5510	
Business address: Interdepartmental address: MD 221		
Home phone number: (	Home fax number: ( ) -	
Home address: 6817 Queens Ferry Rd. Baltimore, MD 21239		
Citizenship: China	Social Security Number:	
Are you a Howard Hughes Medical Institute employee or investigator? Are you a Kennedy Krieger Institute employee or investigator?	☐ Yes ☒ No ☐ Yes ☒ No	

JHU REF: 4485

INVENTION DESCRIPTION

Describe the invention completely, using the outline given below. Please provide an **electronic copy** of the invention disclosure document, references, and abstracts in Windows format on CD-ROM or floppy disk if possible

Brief Description

Mucus Barriers cover all routes of entry into the body, including the gastrointestinal tract, nose, lungs, and female reproductive tract. It is critical in many applications that drug or gene delivery particles be able to cross mucus barriers efficiently to ensure the effective delivery of their therapeutic payload to underlying cells. Other applications include diagnostics, imaging, etc.

To reduce interactions of particles with mucus, we used poly(ethylene glycol) (PEG), a non-mucoadhesive polymer, to surface-modify nanoparticles formulated from polyethylenimine (PEI), dioleyltrimethylammoniumpropane/dioleyl-*sn*-glycerophosphoethanolamine (DOTAP:DOPE), and polysebacic anhydrides (pSA). Standard polystyrene (PS) particles were also modified with PEG as controls.

Using the method of real-time multiple particle tracking (MPT), we measured the mean squared displacements (MSD) of individual polystyrene particles and polyethylenimine gene carriers in mucus from patients with cystic fibrosis, bovine cervical mucus, or synthetic mucus that accurately mimics lung, GI, or vaginal mucus and quantified the effects of surface modification with PEG on the rate of particle transport. We also surface-modified particles with other proteins, DNA, and polymers, and we found that the transport rates were increased.

The average rate of transport of PEI or standard-sized polystyrene (PS) particles surface-modified with PEG increased by approximately 10-fold. Furthermore, the percentage of particles undergoing diffusive transport in mucus, as determined by measuring the linearity in the slope of the MSD, was increased for PEI particles modified with PEG by approximately 10%.

Using confocal microscopy, we determined the rate at which fluorescent nanoparticles (PS, PEI, pSA, DOTAP:DOPE) added to the surface of mucus fluids move into the bulk fluid. We found that surface-modified PEG particles moved much more rapidly into the bulk fluid. Again indicating that surface-modification with PEG increases transport rates in mucus.

JHU REF: 4495

SOFTWARE – Does this disclosure include a software element or software is implemented in the invention ☐ Yes ☒ No
If yes, please complete the Software Information Form which can be found at:
BIOLOGICAL MATERIAL – Does this disclosure include biological material ☒ Yes ☐ No
If yes, please attach a list of materials for reference. A Tangible Property Report of Invention form may be completed if the disclosure is biological materials only. You can find this form at: http://www.ltd.jhu.edu/For%20Hopkins%20Inventors/reporting.html#ROI

2. Problem Solved

Drug and gene carrying nanoparticles delivered to mucus-covered cells in the lung, nose, gastrointestinal, and vaginal tracts must traverse mucus to reach cellular targets. Inefficient particle delivery to those tissues has been attributed to slow transport and/or instability of nanoparticles in mucus. Particles, such as those disclosed herein, that remain stable in mucus and move rapidly through this barrier can be used to deliver therapeutic drugs, chemotherapeutic agents, genes and vaccines, and may be useful in imaging and diagnostic applications in mucosal tissues.

3. Novelty [Identify those elements of the invention that are new when compared to the current state of the art]

(A) Idea that surface modification with PEG can be used to alter the surface chemistry of different types of particles and that each alteration will produce particles that more readily cross mucosal barriers.
(B) Fact that surface modification with PEG renders the particles less adhesive with mucus.
(C) Fact that surface modification with PEG increases particle stability. This leads to the production of more stabile gene or drug carriers.
(D) Fact that cell-specific ligands can be added to PEG to direct targeting of PEG-modified particles to specific cells. This may facilitate delivery of chemotherapeutic agents or targeting to specific tissues through the blood or types of cells in mucus, such as M-cells (immune cells) in the gut.
(E) Fact that mucolytic agents can be used to increase the transport rates of particles in cells and mucus

Importantly, this is shown with PEG, but could be extended to any molecule that renders particles less muco-adhesive as compared to the parent particle.

4. Potential Commercial Use – [What products can be produced with this invention.]

(A) All forms of mucosal drug delivery, imaging, diagnostics, etc.
(B) Same as (A), except in other tissues where enhanced transport rates of particles improves the outcome (e.g., improved drug or imaging agent distribution in a target tissue or organ, including those not related to mucus barriers)
(C) Cell-specific and/or sustained delivery of chemotherapeutic agents for treatment of cancers affecting mucus-covered and other tissues. May be especially useful for: lung, gastrointestinal, bladder, vaginal, and colon cancers.
(D) Delivery of gene therapeutics to mucus-covered and other tissues. For example, delivery of CFTR genes to the lungs or intestines of patients with cystic fibrosis.
(E) Targeting drugs/vaccines to cells in the gastrointestinal tract.

8. Workable Extent/Scope [Describe the future course of related work, and possible variations of the present invention in terms of the broadest scope expected to be operable; if a ***compound,*** describe substitutions, breadth of substituents, derivatives, salts etc., if ***DNA or other biological material,*** describe modifications that are expected to be operable, if a ***machine or device,*** describe operational parameters of the device or a component thereof, including alternative structures for performing the various functions of the machine or device]

Particles that cross mucus barriers more efficiently should find use in dozens of drug therapies (ranging from small molecule therapeutics like chemotherapeutics, to peptides, proteins, oligonucletoides, DNA, etc.). They should also be useful for imaging and diagnostics. Any molecule that, when adsorbed, covalently-attached, or otherwise colocalized with our particles that causes the particles to adhere less to mucus constituents should allow enhanced transport of the particles through mucus barriers. Such molecules are likely to include a variety of proteins, surfactants, sugars, DNA, polyethylene glycol, etc. Importantly, any molecule, therapeutic, diagnostic, etc., may be concentrated within the particles, or on the particle surfaces, for efficient transport through mucus barriers (e.g., in the lungs, nose, gastrointestinal tract, female reproductive tract, etc.) to underlying tissues. The entrapped molecules can then be released over prolonged times at predetermined rates. The enhanced transport rates of these particles in mucus are not likely to be exclusive to mucus, but more likely to any biological environment. The reason is that the coating of the particles can be readily changed to reduce particle adsorption to other biological structures that would slow down particle transport rates. Examples include the extracellular space within tissues and the interior of cells. In other words, coating of particles with certain molecules, such as but not limited to PEG, can lead to more rapid particle movement within any biological environment. This ROI describes several distinct particles (polystyrene, polyethylenimine, liposomes, PLGA, and polyanhydrides) that all transport much more rapidly through mucus barriers when they are modified on their surface with a molecule that makes the parent particle less adhesive to mucus, such as PEG or DNA.

The addition of ligands to the surface of the particles (e.g., by adsorption or covalent attachment) can enhance their specific interaction with target organs, tissues, or even particular cells within a given organ or tissue. It can also enhance particle uptake by specific cells. An example of this is transferrin added to the surface of our mucus-resistant PLGA-DDAB-DNA particles described herein.

Among the countless potential applications, these particles are being/will be studied in the Hanes lab for gene therapy and localized chemotherapy delivery to treat cancer. Asthma treatment, delivery of agents to prevent or treat STDs, etc. are also obvious choices.

Agents that alter mucus, such as mucolytic agents (rhDNase, N-acetylcysteine, etc.), may enhance particle transport through mucus. These agents can be delivered as a bolus prior to particle administration, concomitant to particle administration, or they may be delivered from the particle (encapsulated within or colocalized to the surface, etc.). NAC treatment of mucus, for example, is shown to significantly increase particle transport rates in mucus, which leads to enhanced particle access to and uptake by underlying cells. This is shown herein with liposome particles, polystyrene particles, and PLGA-DDAB-DNA-Transferrin particles.

Something that   seems logical, is to deliver mucoadhesive particles that contain mucolytic agents either prior to or concomitant with the therapeutic/diagnostic/imaging particles. The mucolytic particles would presumably adhere to mucus and degrade it, which would sterically prevent the "therapeutic" particles from adhering and also reduce the mucus barrier by degrading its constituents somewhat. Particles could be modified with molecules that, in this case, promote mucoadhesion (opposite of our "therapeutic" particles).

References [Please cite relevant journal citations, patents, general knowledge or other public information ated to the invention and distinguish between references that (A) contain a description of the current invention m those that (B) contains background information.]

:wson M, Krauland E, Wirtz D, Hanes J, Transport of polymeric nanoparticle gene carriers in gastric mucus, *ɔtech Prog*, published online, [Impact 1.73]
ıwson M., Wirtz D., and Hanes J. (2003) Enhanced viscoelasticity of human cystic fibrotic sputum correlates th increasing microheterogeneity in particle transport, *J. Biol. Chem.*, 278:50393-50401. [Impact 6.70]
ıwson M, Wirtz D, and Hanes J, Real-time nanoparticle tracking in cystic fibrotic sputum treated with mucolytic ents, *J. Aero. Med.*, revised. [Impact 0.97]
ı J, Fiegel J, Krauland E, Hanes J (2002) New polymeric carriers for controlled drug delivery following halation or injection, *Biomaterials*, 23: 4425-4433. [Impact 3.01]; {Times Cited 5}
ı J, Fiegel J, Hanes J, Synthesis and characterization of PEG-based ether-anhydride terpolymers: New polymers r controlled drug delivery, *Macromolecules*, under revision.
egel J, Fu J, Hanes J. (2004) Poly(ether-anhydride) dry powder aerosols for controlled drug delivery in the lungs, *Controlled Release*, 96:411-423. [Impact 3.13]
ıh J, Wirtz D., Hanes J (2003) Efficient Active Transport of Gene Nanocarriers to the Cell Nucleus, *Proc. Natl. cad. Sci.*, 100: 3878-3882. [Impact 10.70]; {Times Cited 2}

☐ No references available at this time.

JHU REF: 4425

SECTION B. JHU INVENTOR CERTIFICATION and ASSIGNMENT

This section is to be completed only by those JHU personnel subject to The Johns Hopkins University Intellectual Property Policy. Non-JHU Inventors, and HHMI or KKI Inventors at JHU are subject to a separate assignment and must complete Section C. JHU Inventors who believe they are not subject to The Johns Hopkins University Intellectual Property Policy for the invention described herein must complete Section C.

I/we, the Inventors, hereby certify that the information set forth in this Report of Invention is true and complete to the best of my/our knowledge.

I/we, the Inventors, hereby certify that I/we will promptly advise LTD of any commercial interest regarding the invention described herein.

I/we, the Inventor(s), subject to The Johns Hopkins University Intellectual Property Policy and not under an obligation to assign intellectual property rights to another party, hereby affirm that in consideration for The Johns Hopkins University's evaluation of commercial potential and a share of income which I/we may receive upon commercialization of my/our invention, on the date of my/our signature(s) as indicated below do hereby assign and transfer my/our entire right, title and interest in and to the invention described herein unto The Johns Hopkins University, its successors, legal representatives and assigns.

JHU Inventor Signature	Justin Hanes Typed or Printed Name	Date % of Contribution
JHU Inventor Signature	Michelle Dawson Typed or Printed Name	Date % of Contribution
JHU Inventor Signature	Denis Wirtz Typed or Printed Name	Date % of Contribution
JHU Inventor Signature	Jie Fu Typed or Printed Name	Date % of Contribution
JHU Inventor Signature	Typed or Printed Name	Date % of Contribution
The Johns Hopkins University Intellectual Property states that inventorship contribution is equal unless otherwise agreed upon. Inventorship contribution provides for potential revenue and cost sharing. JHU inventor's percentages must TOTAL 100%.	**JHU INVENTOR'S TOTAL** 100 %	

ROI Supporting Information and Results

Modification of Particle Surface Properties with Non-Mucoadhesive Polymers, including PEG. High MW poly(ethylene glycol) (PEG) has been used as a mucoadhesive added to polymeric systems for its reported ability to interpentrate into the mucus network[1-4] and hydrogen bond to mucins [5-7]. However, we have found that modifying the surface of different particle types formulated from polymers and liposomes with PEG decreased the adsorption of mucus components to the particle surface and allowed more rapid transport through mucus with a reduced number of adhesive particles (see summaries of our findings).

Modification of particle surface with other polymers, proteins, or non-mucoadhesive materials may also result in increased transport in mucus and other adhesive biological fluids, such as serum. In support of this hypothesis, we have previously shown that modification of particle surface by the adsorption of hydrophilic DNA to the surface of hydrophobic PLGA-DDAB nanoparticles increases transport in mucus[8]. Additionally, we found that non-specific adsorption of polylysine (PLL) or bovine serum albumin (BSA) to hydrophobic polystyrene particles increases transport in mucus (refer to Proof of Principle). Our findings also indicate that the attachment of PEG to the surface of particles formulated from a variety of materials greatly reduces the effects of mucin adsorption, increases transport rates, and provides increased particle stability. Other molecules such as surfactants or polymers, including poly (aspartic acid), and proteins, such as heparin, may also increase transport rates in mucus.

Surface Modification of Different Core Particles with PEG. PEG moieties were conjugated onto the surface of PEI/DNA nanocomplexes at different N/P ratios and PEG concentrations. Transport rates of PEI/DNA complexes with N/P ratio of 20 (unmodified) and modified with 10% PEG concentration were quantified using multiple particle tracking techniques.

PEG moieties were non-specifically adsorbed to the surface of 500-nm polystyrene particles using a standard adsorption protocol accessible through Polysciences (protocol can be attached). The transport rates of control particles and surface modified polystyrene particles (modified with PEG 3000, PLL, or BSA) were measured with multiple particle tracking techniques. One dimensional diffusivity of unmodified polystyrene particles was also determined with time-lapsed confocal microscopy.

Liposomal formulations of DOTAP:DOPE were also modified to include PEG. DOPE:PEG-2000 was combined with a cationic lipid-based tranfection reagent, DOTAP:DOPE , and a fluorescent

lipid, NBD:DOPE,. at ratios of 1:48:1, respectively. Briefly, cationic lipids were combined and dissolved in 1:1 chloroform/ methanol mixture (50 mM lipid composition) and rotary evaporated. The liposomes were resuspended by shaking the film in 20 mM Hepes buffer at 4° C for 24 hours, sonicating in 30 second pulses for 10 intervals, and filtering solutions with 0.4 μm Whatman filter. Liposomes were complexed with DNA at a 1:1 ratio, final DNA concentration was 25 μg/ml DNA. Reduced adhesion of particles in mucus was assayed by confocal microscopy and laser Doppler anemometry.

Information regarding the preparation of Poly(sebacic) acid anhydride and modification with PEG can be found in the Patent Application and referenced works and cited paper [9].

Relevant Applications for Increased Mucoadhesion. Particles with surface chemistries that favor interaction with mucus, including more hydrophobic or highly-charged particles, can be used as mucoadhesive particles to target drugs, contraceptives, or other products to mucus or biomacromolecules that are adhesive with the modified particle surface.

Effects of Surface Modification of Particles with PEG on the Rate of Particle Transport in Mucus Quantified with Real-Time Multiple Particle Tracking. MPT is a powerful method for studying particle transport rates since this method allows us to simultaneously measure the transport rates of hundreds of individual particles in real time. Our lab uses MPT to quantify gene carrier transport through CF mucus [10] and through the cell [11]. Particles that can effectively transport through mucus must be able to move through the mucus mesh with minimal interactions. To do this effectively, we believe they must be small and resistant to adsorption to mucus.

Proof of Principle #1: PEG-Polystyrene Particles

The effect of surface modification with PEG on transport rates of polystyrene particles in mucus were quantified with multiple particle tracking (Fig. 1). This data shows that PEG modification can greatly enhance the diffusion of PS particles into mucus (Fig. 1).

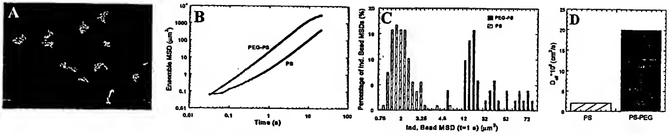


Figure 1. Multiple particle tracking (MPT) was used to quantify effects of surface modification by PEG adsorption on polystyrene (PS) particle transport rates in bovine cervical mucus. **(A)** Tracking the mean squared displacements (MSDs) from particle trajectories with real-time MPT and epifluorescence video microscopy. **(B)** MSDs of PS particles vs. PEG-modified PS particles showing that PEG-modified particles move one to two orders of magnitude more quickly through mucus. **(C)** The distribution of the individual bead MSDs at t=1 s further demonstrates the vastly improved transport rates of PEG-modified particles. **(D)** Effective diffusivities (t=1s) of PS particles vs. PEG-modified PS particles.

Proof of Principle #2: PEG-Polyethylenimine/DNA Nanoparticles

The effect of surface modification with PEG on polyethylenimine transport rates in CF mucus was quantified with multiple particle tracking (Fig. 2). PEG was covalently attached to PEI/DNA particles. PEG modification greatly enhanced the diffusion of PEI gene carriers into mucus (Fig. 2).

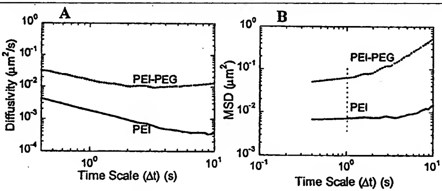
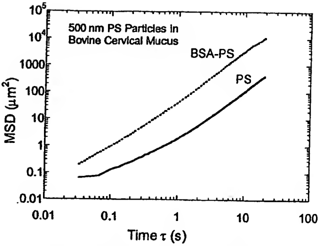


Figure 2. Multiple particle tracking (MPT) was used to quantify effects of surface modification by PEG of PEI/DNA particle transport rates in mucus. (A) Effective diffusivities (t=1s) of PEI particles vs. PEG-modified PEI particles (B) MSDs of PEI particles vs. PEG-modified PEI particles showing that PEG-modified particles move much more quickly through mucus.

Proof of Principle #3: BSA-Polystyrene Particles

BSA-coated particles move more rapidly in mucus than PS (Fig. 3), suggesting that coating particle surface with other molecules, proteins, or polymers, may also increase transport in mucus.

Figure 3. Effect of surface-modification of PS particles with BSA on the rate of particle transport in bovine cervical mucus. PS particles are 500-nm in diameter and the transport rates is assayed by multiple particle tracking (shown is the ensemble average MSD of 60-80 particles for each).



__*Additional Details on Determining the Mean Squared Displacement, Diffusion Coefficient, and Mode of Transport of Particles as Measured by Multiple Particle Tracking (MPT).*__ Images of particles are acquired as described [12]using a SIT camera (VE-1000 Dage-MTI, Michigan City, IN) mounted on an inverted epifluorescence microscope maintained at 37 C and equipped with a 100-x magnification, 1.3 numerical aperture, oil-immersion lens (Nikon, Melville, NY). These images are analyzed using a custom subroutine incorporated to the software Metamorph (Universal Imaging Corp., West Chester, PA). The displacements of the centroids of individual microspheres are simultaneously tracked in the focal plane of the microscope for 20 s at a rate of 30 Hz, as many times as necessary to monitor a total of ~100 particles for each tested specimen. Our software can track hundreds of particles simultaneously, but the density of the microspheres was adjusted to limit the number of probe particles to 10-30 per field of view in order to reduce potential correlated interactions between neighboring particles. The spatial resolution, which was evaluated by tracking the apparent displacement of latex beads firmly tethered to the coverslip, was ~ 5 nm [12]. From the trajectories of the microspheres centroids, individual time-lag-averaged mean squared displacements (MSD), $<\Delta r^2(t)>$, are computed [13], from which time-lag-dependent MSD distributions and distribution of the diffusion coefficient ($D= <\Delta r^2(t)>/4t$), are generated. These distributions are normalized by the time-lag-averaged, ensemble-averaged MSD and subsequently analyzed by computing median, standard deviation, and skewness, statistical parameters that describe the heterogeneity of transport through the samples. The first proof of principle and more details about the implementation and use of multiple-particle tracking to quantitatively assess the micro-heterogeneity of biopolymer networks can be found elsewhere [12].

Confirmation by Time-Lapsed Confocal Microscopy: PEI-PEG. We used confocal microscopy to measure the apparent diffusional velocity of particles or gene carriers in a mucus slab. With this technique, particles are added to the surface of a mucus slab, and the motion of the particle front into the fluid is assayed by determining the depth of penetration of the particles (in two-dimensional (x,y) image) into the fluid (third dimension (z)). The diffusivity is calculated by one-dimensional diffusion model (Δz^2/total time)

Over a 30 min period PEI/YFP (yellow fluorescent protein DNA) complexes added to the surface of a sputum slab remained in the same x-y plane (slice thickness was 0.37 μm) while PEI-PEG (10%)/YFP complexes translocated over several planes (distance ~ 1 μm) (Fig. 3C). The measured velocity of PEI-PEG/YFP particles was 330 nm/min in 30 min. Velocity of unmodified PEI/DNA carriers was too small to measure. The effective diffusion coefficient of PEG-modified PEI/DNA complexes, obtained by assuming Fickian diffusion, was 2.7×10^{-4} $\mu m^2/s$..

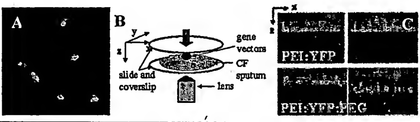
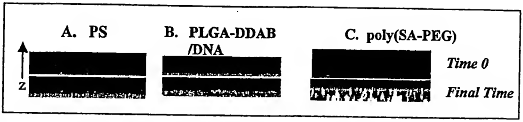


Figure 3. The mobility of PEI/YFP and PEI-PEG/YFP (10% PEG) complexes was determined by 4-D confocal microscopy. (A) Confocal image of PEG-PEI/DNA nanocomplexes following the addition of avidin, NeutraLite Texas Red conjugate. The PEI is labeled with Oregon Green (both dyes from Molecular Probes). Colocalization of red (avidin) and green (PEI/DNA) implies successful conjugation of Biotin-PEG to PEI/DNA nanocomplexes. (B) Fluorescent particles were deposited on the surface of CF sputum, and allowed to diffuse randomly through the specimen. (C) 3-D fluorescent images were recorded at 5-minute intervals (30-minute period) and the 1-D diffusion of complexes was estimated by measuring the translocation of the fluorescent gene carrier front.

Proof of Principle #3 & 4: Poly (SA-PEG) and PLGA-DDAB/DNA versus PS Nanoparticles.

One-dimensional diffusivities of PLGA-DDAB/DNA, poly(SA-PEG), and PS particles in synthetic mucus formulated to model lung mucus were measured using time lapsed confocal microscopy. The diffusivity of PS particles in synthetic mucus was 5.3×10^{-5} μm^2/s. The diffusivity of PLGA-DDAB/DNA particles was 2×10^{-3} μm^2/s. The diffusivity of poly(SA-PEG) in mucus was 2×10^{-2} μm^2/s. Refer to Figure 4. We found that pSA-PEG particles diffused more rapidly than PLGA-DDAB/DNA particles, which diffused more rapidly than PS. Particle sizes upon microscopic observation appeared similar. Increased mobility of pSA-PEG is evidence of the increased mobility of PEG-coated particles.

Figure 4. The mobility of (A) PS, (B) PLGA-DDAB/DNA, and (C) poly(SA-PEG) in synthetic mucus modeling lung mucus was determined by 4-D confocal microscopy.



Additional Details Regarding Determination of the One-Dimensional Diffusivity of Particles in Mucus or Other Fluids Using Laser Scanning Confocal Microscopy. Confocal images of gene vectors in CF sputum are captured with the high performance cooled digital camera AxioCAM HR, attached to a Zeiss LSM 510 Meta laser scanning confocal microscope. The LSM 510 Meta is ideal for time-lapsed 3-dimensional imaging since the microscope is completely motorized and fully automated through LSM software. Subroutines, which allow us to repeat the experimental conditions including the thickness of the z-slice, laser intensity, and time and bleaching intervals, were developed for each application including the FRAP, 4-D mobility, and avidity assays. By allowing the LSM software to control the course of microscopy experiments we are able to repeat experimental conditions with great precision. Particles suspended in solution (10 µl) applied to the surface of a CF sputum sample on a Biophetic cover slide, which is imaged with a LSM 510 Meta confocal - an inverted light microscope (thus vectors are moving into the plane of focus). The cover slide was placed in the Bioptechs thermal regulated chamber and allowed to heat up and equilibrate for approximately 30 minutes prior to imaging. Slice thickness is optimized to increase the depth of focus (~30 µm), so gene vectors that move rapidly can be imaged over a long period of time (90 minutes). Four-dimensional (x, y, z, and t) images of CF sputum slab are collected over a 30-90-minute time period with a time-interval of 5 minutes (Fig. 7). This technique allows us to track the motion of gene vectors and determine an effective velocity and 1D diffusion coefficient of gene vectors in CF sputum - before and after the addition of mucolytic agents. Although this technique does not allow high temporal resolution as seen in MPT, it is an excellent complement for MPT in that it gives us a method of estimating the long-range mobility of gene vectors. We also use this method to focus on immobile beads and to determine changes in mobility after the addition of mucolytic agents (see preliminary results).

Effects of Surface-Modification with PEG on the Adhesion of Particles with Mucus. Mucus adsorption to particle surface results in large changes in the size and zetapotential of particles not modified with PEG (refer to [8] for details on measuring surface charge and particle size). PEG-modified particles had more neutral surface charge and underwent less extreme changes in surface charge upon incubation with mucus, indicating that less mucus adsorbs to the particle surfaces suggesting particles are less adhesive with mucus. Note that the addition of transferrin to the particle surface did not significantly change the surface charge of PLGA-DDAB/DNA particles.

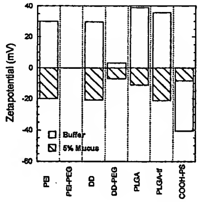


Figure 5. Zetapotentials of nanoparticle gene carriers in 150 mM NaCl and in 1:20 pig gastric mucus [8] / 150 mM NaCl.

Notation and specific information: An N/P ratio of 20 was used for PEI complexes, DD (DOTAP:DOPE), PLGA (PLGA-DDAB/DNA)[8], PLGA-tf (PLGA-DDAB/DNA with Apo-Transferrin)(modified protocol included).

Effects of Addition of Targeting Ligand on the Uptake and Transfection of Surface-Modified Particles by Lung or Gut Cells. Cell-specific targeting ligand was added to the particles to improve their interactions with cells (Fig. 5). The addition of transferrin (Tf) to particle surface improved the interaction with cultured cells owing to high levels of Tf receptor on cell lines.

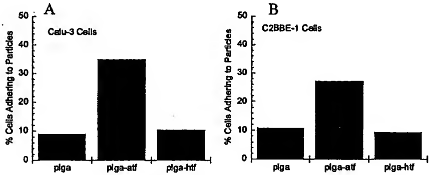
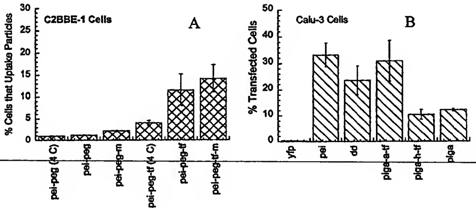


Figure 5. 4° C Adhesion of PLGA-DDAB/DNA NPs modified with apo-transferrin or holo-transferrin to (A) lung and (B) gut cells.

The methods used were modifications of standard assays using flow cytometry to quantify the uptake of fluorescent particles, the adhesion of fluorescent particles to cell surface when particles are maintained at 4 ° C on ice, or transfection of cells with DNA expressing a fluorescent protein.

The uptake of PEI-PEG-Tf particles in gut cells (Fig. 6A) and the transfection efficiency of PEI, DOTAP:DOPE, PLGA-DDAB (with and without Tf) nanoparticles in lung cells (Fig. 6B) was assayed using flow cytometry. PEI-PEG-Tf particles were internalized more efficiently by gut cells than PEI-PEG and had high levels of uptake even in the presence of mucus (Fig 6A). Note that mucus has been shown to restrict uptake and transfection in cells. We found that PLGA-DDAB/DNA with apo-Tf transfected cells more efficiently than particles without Tf or particles with other types of Tf. Note that the uptake at 4° C is a control and indicates that particles are adherent to cell surface and not within cells since internalization of particles requires that cells are maintained at 37 ° C.

Figure 6. (A) Uptake of PEI-PEG particles in gut cells with or without 10% mucus (m) and (B) transfection of lung cells with yellow fluorescent protein (YFP), PEI, DOTAP:DOPE (dd), PLGA-DDAB/DNA with apo-Tf, holo-Tf or no Tf.



Addition of Mucolytic Agents to Mucus Prior to Particle Administration Increases Cell Uptake of Particles. Using concentrations of mucolytic agents used in transport experiments [10, 14] we tested the ability of mucus to increase particle uptake into cells. Addition of mucolytic agents increased uptake of PS particles (Fig. 7A) in Calu-3 (lung) cells and uptake of cationic liposomes (DOTAP:DOPE) (Fig. 7B) into C2BBE-1 (gut) cells.

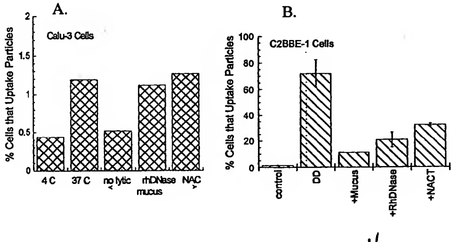


Figure 7. Uptake of (A) 200 nm PS particles in Calu-3

lung cells and (B) cationic DOTAP:DOPE liposomes in C2BBE-1 colon cells is increased by the addition of mucolytic agents.

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All publications, patents and patent applications disclosed herein are incorporated into this application by reference in their entirety.